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Entocybe haastii from Watagans National Park, New South Wales, Australia

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Abstract — *Entocybe haastii* comb. nov. (*Entoloma haastii*) is distinguished by isodiametric minutely rounded pustulate-angular basidiospores, a dark blue black to nearly black pileus that lacks brown tones, dark blue grey lamellae, an appressed fibrillose blackish blue stipe, intracellular pigment in the pileipellis and inflated hyphae in the outer pileal trama, and the faintly parietal pigment on narrow pileal tramal hyphae.

Key words — *Basidiomycota*, *Entolomataceae*, new combination

Introduction

Entocybe T.J. Baroni et al. (*Basidiomycota*, *Agaricales*, *Entolomataceae*) was erected to accommodate a monophyletic group of species with the following set of morphological features: a slender tricholomatoid or mycenoid to collybioid habit, a relatively fragile, appressed fibrillose stipe, small, obscurely angular basidiospores with 6–10 facets (angles) in polar view and undulate-pustulate or rounded pustulate surface ornamentation overall, and a pileipellis subtended by inflated hyphae in the outer pileal trama (Baroni et al. 2011). An agaricoid fungus with this character set recently collected in Watagans National Park in central New South Wales was determined to be *Entoloma haastii*, a new report for mainland Australia. Herein, we recombine *E. haastii* in *Entocybe*.

Materials & methods

Macromorphological and micromorphological features

Techniques and equipment for collecting and describing basidiomata in the field, GPS coordinates, microscopy of dried collection including how basidiospores were

Table 1. Collections used in the phylogenetic analyses.

Species from GenBank	Collection Identifier	GenBank Accession Numbers		
		mtSSU	LSU	RPB2
Calliderma sp.	DLL 10025	JQ793648	JQ793642	—
	DLL 10054	—	JQ793649	—
	DLL 10088	JQ793643	JQ793650	JQ793657
Clitocybe dealbata	HC95cp	AF357138	AF223175	DQ825407
Clitopilopsis hirneola	8490TJB	GU384587	GU384611	GU384646
Cl. hirneola [*Clitopilus hirneolus*]	263	GQ289352	GQ289211	GQ289278
Clitopilus cystidiatus	26	GQ289287	GQ289147	GQ289220
C. prunulus	8229TJB	—	GU384615	GU384650
	9663TJB	GU384586	GU384614	GU384648
C. "*cinerascens*"	8024TJB	GU384585	GU384613	GU384647
Entocybe haastii	**DLL 9868**	**JQ793644**	**JQ793651**	**JQ793658**
	DLL 10087	**JQ793645**	**JQ793652**	**JQ793659**
Entc. haastii [*Entoloma haastii*]	126	GQ289307	GQ289167	GQ289238
Entc. nitida [*E. nitidum*]	7526TJB	GU384602	GU384626	GU384655
	s	GQ289315	—	GQ289246
Entc. nitida [*E. alcedicolor*]	210	GQ289292	GQ289152	GQ289224
Entc. trachyospora [*E. trachyosporum*]	414	GQ289339	GQ289199	—
	405	GQ289338	GQ289198	—
Entc. trachyospora [*R. trachyospora*]	5856TJB	GU384605	GU384629	GU384658
Entc. turbida [*E. turbidum*]	27	GQ289341	GQ289201	GQ289269
	6949TJB	GQ289341	GQ289201	GQ289269
Entc. vinacea [*E. vinaceum*]	8870TJB	GU384598	GU384631	GU384651
E. bloxamii	219	GQ289294	GQ289154	GQ289226
E. caesiolamellatum [*E. bloxamii*]	6117TJB	—	AF261289	—
E. coeruleogracilis [*E. haastii*]	216	GQ289308	GQ289168	GQ289239
	217	GQ289309	GQ289169	GQ289240
E. cretaceum	213	GQ289302	GQ289162	GQ289233
E. gelatinosum	212	GQ289305	GQ289165	GQ289236
E. griseolazulinum	i11	GQ289306	GQ289166	GQ289237
E. indigoticoumbrinum	83	GQ289318	GQ289178	GQ289249
E. kermandii	222	GQ289313	GQ289173	GQ289244
E. perbloxamii	71	GQ289318	GQ289178	GQ289249
E. prunuloides	40	GQ289324	GQ289184	GQ289255
Entoloma sp.	DLL 10217	JQ793646	JQ793653	JQ793660
	BY21	—	AF261309	—
	DLL 10055	—	JQ793655	JQ793662
Lepista ovispora	442	GQ289347	GQ289207	GQ289274
Rhodocybe sp. [*Clitopilus* sp.]	211	GQ289353	GQ289212	GQ289279
R. aureicystidiata	PBM1902	—	AY380407	AY337412
R. caelata [*C. caelatus*]	6995TJB	GU384600	GU384625	GU384652
R. fallax	CBS129.63	AF357083	AF223166	EF421051
R. fallax [*C. fallax*]	262	GQ289349	GQ289210	GQ289275
	37	GQ289350	GQ289276	GQ289276
R. mundula	7599TJB	—	AY700182	DQ474128
R. nitellina [*C. nitellinus*]	265	GQ289354	GQ289214	GQ289281
	400	GQ289355	GQ289215	GQ289282
R. pallidogrisea [*C. pallidogriseus*]	118	GQ289356	GQ289216	GQ289283
R. popinalis [*C. popinalis*]	260	—	GQ289213	GQ289280
R. popinalis	6378TJB	GU384601	AF261285	GU384654
R. pruinosostipitata	MCA1492	GU384608	GU384627	GU384653
R. pseudopiperita [*C. pseudopiperitus*]	162	GQ289357	GQ289217	GQ289284
R. spongiosa	MCA2129	GU384604	GU384628	GU384657
Tricholoma vaccinum	446	GQ289358	GQ289219	GQ289286
T. viridiolivaceum	PBM3093	—	JF706317	JF706319

New sequences generated for this study are shown in bold. Square bracketed annotations indicate species names applied in GenBank that differ from those in the phylogram (Fig. 1).

Plate 1. *Entocybe haastii* {DLL 10087}. A: Densely appressed-fibrillose pileus surface; B: Dark bluish-grey lamellae and stipe apex. Bar (A, B) = 5 mm.

measured, and digital microphotographs have been described in Largent et al. (2011a,b), while techniques for describing, coding, naming colors from Kornerup & Wanscher (1978) and factors determined from mathematical analyses in the descriptions are covered in Largent et al. (2013). Generic abbreviations used are: *E.* = *Entoloma*; *Entc.* = *Entocybe*.

The holotype collection and one additional collection are deposited in The Plant Pathology Herbarium, Orange Agricultural Institute (DAR). The herbarium acronym is from Thiers (2012).

DNA sequences

Sequences were obtained for three loci (mtSSU, LSU, RPB2) for two *E. haastii* collections from Australia (Table 1). The extraction and Polymerase Chain Amplification (PCR) protocols for the mitochondrial small subunit of the ribosomal DNA (mtSSU), variable domains (D1, D2) of the nuclear large subunit (LSU) and the second largest subunit of the RNA polymerase gene (RPB2) follow Largent et al. (2011b). The primers rpb2-i6f and rpb2-i7r or rpb2-EntF2 and rpb2-EntR4 were used to amplify the RPB2 (Co-David et al. 2009, Largent et al. 2013). Sequences were generated on an Applied

Biosystems 3130xl Genetic Analyzer at Middle Tennessee State University using the sequencing protocols outlined in Largent et al. (2011b).

Sequences were assembled and edited using Sequencher ver. 4.2.2 (Gene Codes Corporation, Ann Arbor, MI) and multiple sequence alignments were generated using MAFFT version 7.1 (Katoh & Standley 2013) and adjusted manually using Se-Al ver. 2.0a11 Carbon (Rambaut 2002) after exclusion of introns from the RPB2 alignment. The combined mtSSU, LSU and RPB2 sequences were aligned with closely related sequences from GenBank within the Prunuloides clade, representatives of the basal Rhodocybe-Clitopilus clade, and appropriate outgroups (Matheny & Baroni 2011) (Table 1). The alignment lengths were 1909 bp (mtSSU), 1472 bp (LSU), and 972 bp (RPB2).

The sequence alignment was analyzed using a Maximum Likelihood (ML) search GTRGAMMA model using RAxML-HPC v. 7.2.8 ALPHA (Stamatakis 2006). The dataset was partitioned using 4 partitions: RPB2 (across each codon position), mtSSU and LSU. Bootstrap proportions were estimated based on 1000 rapid ML replicates and 1000 bootstrap replicates (Stamatikas et al. 2008).

Results

The best likelihood tree in RaxML showed that species identified as *Entocybe* (Baroni et al. 2011) formed a well-supported clade (BS = 97, Figure 1) and lends further support for the generic concept as defined by Baroni et al. (2011). Additional comments on *Entc. haastii* are provided in the Comments section.

Taxonomy

Entocybe haastii (G. Stev.) Largent, **comb. nov.** Plates 1–3

MycoBank MB 804015

≡ *Entoloma haastii* G. Stev., Kew Bull. 16(2): 229 (1962).

Habit mycenoid but with a broad stipe base. Pileus 24–33 mm broad, 10–11 mm high, umbo obscurely broad, opaque, not hygrophanous, not translucent-striate, dull, moist but not sticky, conic- or convex-campanulate, densely appressed- or matted-fibrillose, rivulose, dark blackish blue (19F3 or 19F3–4) to nearly black, lacking any brown color, becoming silky or satiny appressed fibrillose with maturity; margin decurved, entire becoming slightly eroded; context 0.75–2.0 mm thick above the stipe, ± violaceous. Odor mild or faintly fragrant. Taste mild, at times suggestively sweet. Lamellae 11–15 mm long, 5.5–11.0 mm broad, narrowly adnate or adnate, close, at first dark bluish grey (19E-F2–3) with basidiospore maturity pale orange (6A3) then orange grey (6B2); margin smooth and concolorous. Stipe 40–65 3–5 (apex) – 4–10 mm (middle) – 6–12 (base) mm, clavate, shiny and glossy, appressed-fibrillose, blackish blue but somewhat lighter than the pileus (19E5 or 20F6), white or yellowish white at the base, hollow and splits longitudinally with ease; context cartilaginous, white at least at the base; basal tomentum absent. Bruising reactions none.

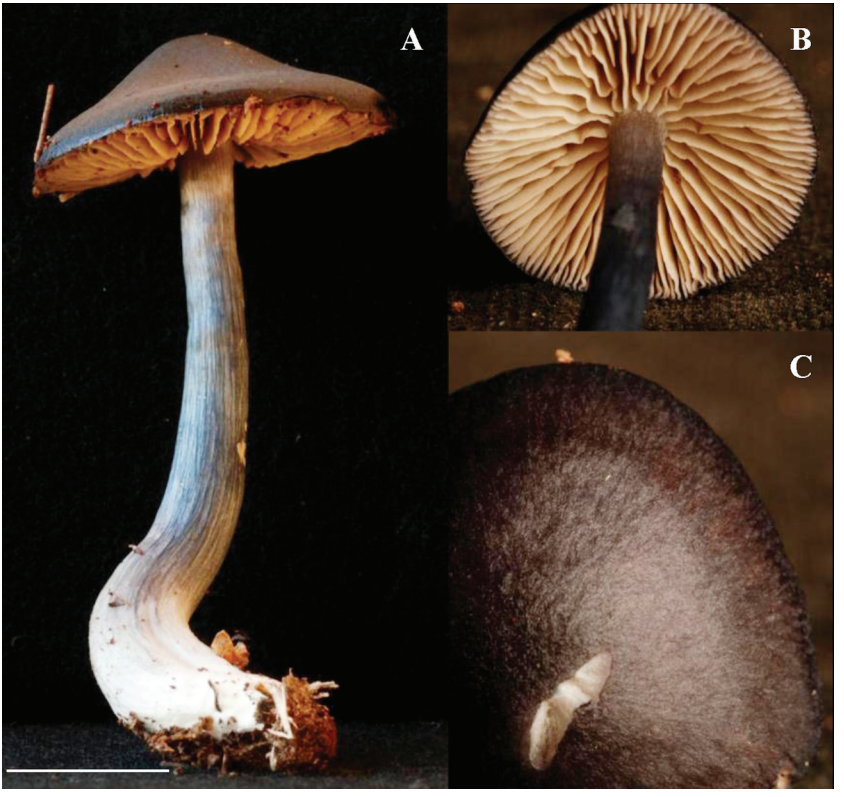

Plate 2. *Entocybe haastii* {DLL 9868 A, B; DLL 10087 C}. A: Stature; B: Mature lamellae; C: Mature pileal surface. Bar for A, B = 15 mm; C = 10 mm.

Basidiospores in squashed mounts of lamellae in 3% KOH often collapsed and thin-walled, some distinctly angular and some indistinctly to faintly rounded pustulate, in mounts from stipe surface decidedly rounded pustulate with 6 or more facets, in profile view isodiametric to subisodiametric, rarely heterodiametric, in polar view isodiametric 6.4–9.5(–10.2) × 5.5–7.9(–9.0) µm (x = 7.7 ± 0.7 × 6.9 ± 0.6 µm; E = 0.96–1.31, Q = 1.11 ± 0.07 (isodiametric); n = 90/2). Basidia cylindro-clavate, hardly tapered, 26.2–39.8 × 7.7–11.7 µm (x = 32.1 ± 3.8 × 9.5 ± 1.0 µm; E = 2.51–4.42, Q = 3.41 ± 0.48; n = 26/2); 2– or 4–sterigmate. Hymenial cystidia absent. lamellar tramal hyphae subparallel, moderately short and broad, 48.2–131.3 × 7–30.2 µm. Pileipellis mostly a multi-layered cutis with a few semi-erect terminal cells, subtended by inflated cells of the pileal trama. Pileocystidia (terminal cells) cylindric to narrowly obclavate, 54.1–81.1 × 2.5–7.8 µm (n = 7/1). Pileal tramal hyphae beneath the pileipellis, short, broad and inflated, 47–129 × 8–22 µm

enlarging up to 188 µm long and 33 µm wide near the hymenium (n = 14/2). Stipitipellis a cutis. caulocystidia and hymenial clusters absent. oleiferous hyphae rare to scattered in the pileal trama. Lipoid globules absent. Brilliant granules absent. Pigments cytoplasmic, brownish blue, soluble in 3% KOH in pileipellis and inflated hyphae of outer pileal trama; faintly parietal or possibly encrusted on the narrow hyphae of the pileal trama. Clamp connections present on hyphae of all tissues.

Ecology and distribution — solitary in soil among leaf litter or in mosses on a rock, warm gallery rainforest, Watagans National Park, New South Wales.

collections examined – **AUSTRALIA. New South Wales**, Central Hunter District, the Watagans National Park, Boardinghouse Dam Track, 32°59′58.6″S 151°24′15.5″E, 13 April 2010, DL Largent 9868 (dar); 32°59′56″S 151°24′15″E, 12 April 2011, DL Largent 10087 (dar).

Comments — *Entocybe haastii* from New South Wales lacks the brown color in the pileus reported for this species from Tasmania and New Zealand (Noordeloos & Gates 2012). The lamellae from Tasmania are whitish while those from Australia and New Zealand are bluish-gray (Horak 2008, Noordeloos & Gates 2012). This variability suggests that fine color distinctions are not diagnostic for *Entc. haastii.*

Similar to *Entc. haastii,* both *Entc. nitida* (Quel.) T.J. Baroni et al. (≡ *Entoloma nitidum* Quél.) from Europe and *Entoloma alcedicolor* Arnolds & Noordel. from Holland have small isodiametric basidiospores and abundant clamp connections and lack cheilocystidia (Noordeloos 2004).

Entocybe nitida is distinguished from *Entc. haastii* by a glabrous or radially fibrillose pileus, white 30-100 mm long immature lamellae, ≤100 mm long cylindrical stipe, slightly farinaceous or raphanoid odor, and shorter and broader tramal cells subtending the pileipellis (Noordeloos 1981, 1992, 2004). Although Noordeloos (1992) identified basidiomata from Germany with bluish lamellae as *Entc. nitidum,* identification remains speculative as no collection was retained.

Entoloma alcedicolor differs from *Entc. haastii* by its steel blue pileus and stipe, garlic odor, squamulose pileus center, and trichodermial pileipellis on the center of the pileus.

Entoloma gracilior Noordel. & G.M. Gates from Tasmania, which shares with *Entc. haastii* black pilei, lilac grey lamellae, a dark blue stipe as well as a similar pileipellis and basidiospores, differs in its more mycenoid stature and more slender (2–4 mm across the apex) equal stipe, a weakly hygrophanous smooth pileus with a translucent-striate margin, and heterodiametric basidiospores (Noordeloos & Gates 2012).

Entoloma coeruleogracilis G.M. Gates & Noordel., also from Tasmania, is distinguished by its sky blue stipe and pileus with a violet hue and the 1–3 mm broad cylindrical stipe.

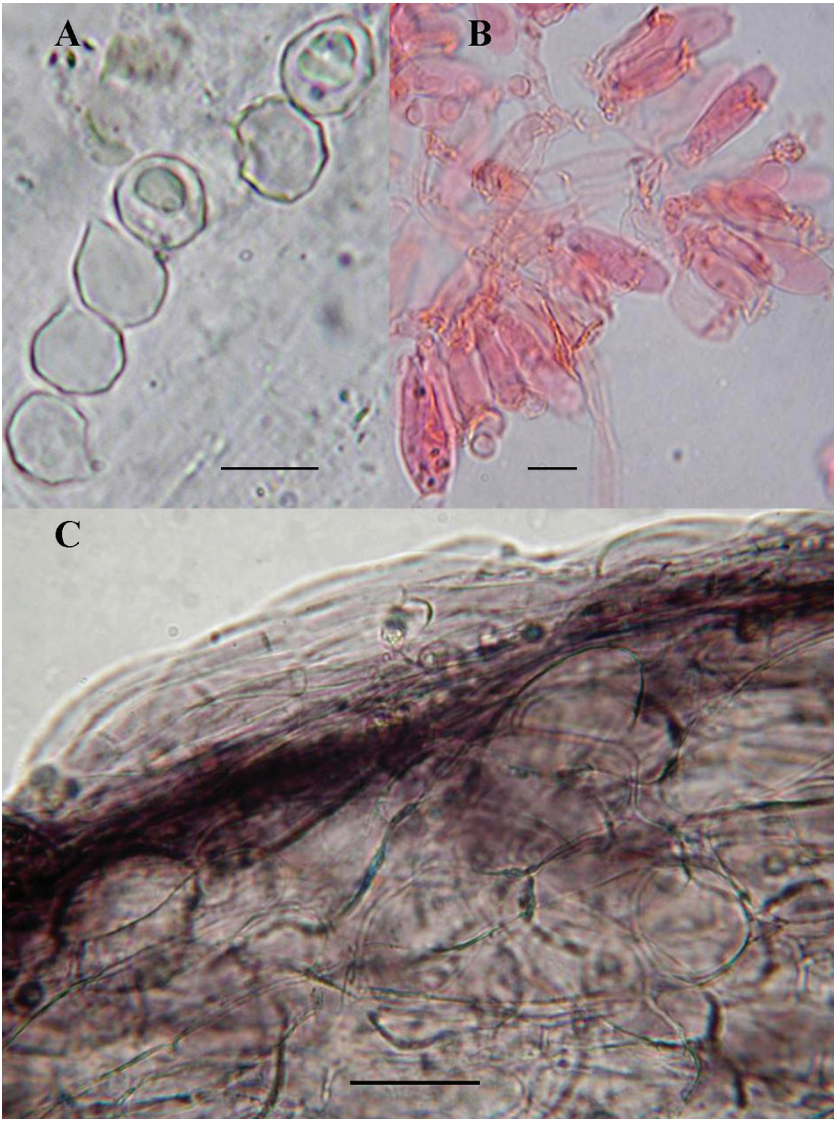

Plate 3. *Entocybe haastii* {DLL 10087}. A: Basidiospores from stipe surface; B: Basidia and basidioles; C: Pileipellis and subtending inflated cells in pileal trama. Bars A = 7.0 µm; B = 10 µm; C = 22 µm.

Our phylogenetic analysis (Fig. 1) clearly supports (BS = 97) *Entc. haastii* within *Entocybe* as described by Baroni et al. (2011). *Entocybe haastii, E. coeruleogracilis, Entc. nitida,* and *E. alcedicolor* segregate as a sub-clade with

moderate support (BS = 77). Noordeloos & Gates (2012) classify these taxa in *Entoloma* sect. *Nitida* (Romagn.) Noordel. based on a number of morphological features.

Acknowledgments

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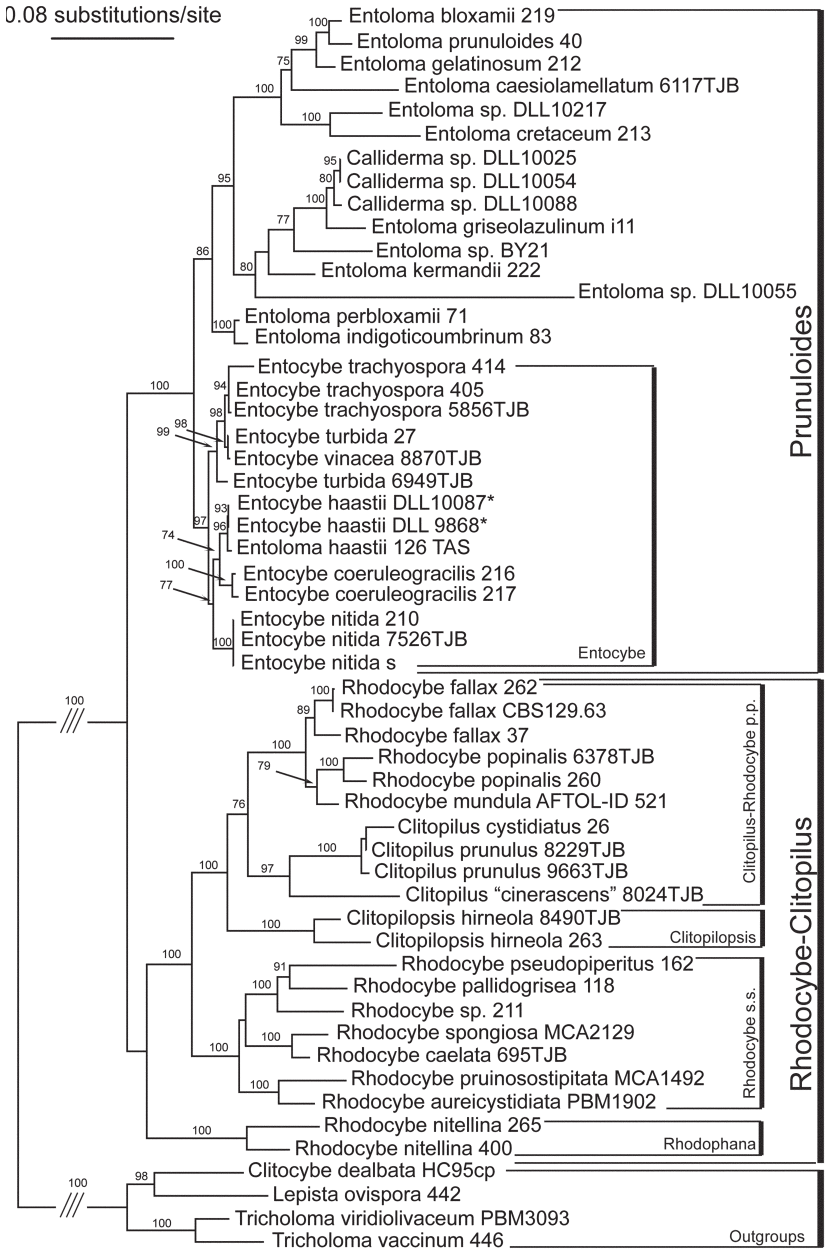
Figure 1. Maximum likelihood tree (LSU+mtSSU+RPB2) showing the position of *Entocybe haastii* from Australia (AUS*) and Tasmania (TAS) relative to other *Entocybe* collections. Each sequence is labeled with the GenBank-listed isolate number. Branches with >70% support from 1000 rapid bootstraps provide statistical support. *Clitocybe dealbata*, *Lepista ovispora*, *Tricholoma vaccinum*, and *T. virdioviolaceum* serve as outgroups. Three slanted lines denote truncated branches.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Calliderma sp. DLL10025
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Calliderma sp. DLL10088
Entoloma griseolazulinum i11
Entoloma sp. BY21
Entoloma kermandii 222
Entoloma sp. DLL10055
Entoloma perbloxamii 71
Entoloma indigoticoumbrinum 83
Entocybe trachyospora 414
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Entocybe trachyospora 5856TJB
Entocybe turbida 27
Entocybe vinacea 8870TJB
Entocybe turbida 6949TJB
Entocybe haastii DLL10087*
Entocybe haastii DLL 9868*
Entoloma haastii 126 TAS
Entocybe coeruleogracilis 216
Entocybe coeruleogracilis 217
Entocybe nitida 210
Entocybe nitida 7526TJB
Entocybe nitida s
Entocybe
Prunuloides
Rhodocybe fallax 262
Rhodocybe fallax CBS129.63
Rhodocybe fallax 37
Rhodocybe popinalis 6378TJB
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Rhodocybe mundula AFTOL-ID 521
Clitopilus cystidiatus 26
Clitopilus prunulus 8229TJB
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Clitopilus “cinerascens” 8024TJB
Clitopilus-Rhodocybe p.p.
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Clitopilopsis
Rhodocybe pseudopiperitus 162
Rhodocybe pallidogrisea 118
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Rhodocybe spongiosa MCA2129
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Rhodocybe pruinosostipitata MCA1492
Rhodocybe aureicystidiata PBM1902
Rhodocybe s.s.
Rhodocybe nitellina 265
Rhodocybe nitellina 400
Rhodophana
Rhodocybe-Clitopilus
Clitocybe dealbata HC95cp
Lepista ovispora 442
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Tricholoma vaccinum 446
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